

WHEN SENSE MAKES SENSE

EPIGENETIC REGULATION BY EARLY ADVERSE EXPERIENCES IN THE NICU

BIOCHEMICAL PATHWAYS TO PRETERM INFANTS' OUTCOMES



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May 9th 2019 Queen Elizabeth University Hospital, Glasgow



Presentation Scientific Institute IRCCS E. Medea



Neurorehabilitation hospital ruled by the Italian Ministry of Health

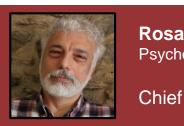
Infants with <u>neurodevelopmental disabilities</u> (cerebral palsy, genetic syndromes, severe prematurity, psychomotor delay, pediatric tumors)







Presentation 0-3 Center - Team members



Rosario Montirosso, Pl Psychologist, Psychotherapist

Livio Provenzi, Pl Psychologist, Psychotherapist, PhD

Behavioral Epigenetics Mother-infant interaction



Eleonora Mascheroni Psychologist, PhD

Functional MRI Maternal touch



Lorenzo Giusti Psychologist, Psychotherapist, PhD candidate

Early Intervention Parental Support



Eleonora Visintin Psychologist, Psychotherapy trainee, PhD

Genetic syndromes Mirror neurons



Elisa Rosa Psychologist, Psychotherapy trainee

Early Intervention Behavioral epigenetics



Elena Guida Psychologist

Early Intervention Technological applications





OVERVIEW

A. Preterm birth and NICU stress

B. Behavioral epigenetics principles

C. Preterm Behavioral Epigenetics Project

D. Take-home messages



Very preterm infants in the NICU

A summary of exposures, for bad and for good

Very preterm (VPT) birth (gestational age < 32 weeks) is a major risk factor for human infants development (Blencowe et al. ven in absence of severe neurobehavioral injuries or morbidities, VPT infants are long-lasting nospitalized in th

NICU-related stress

NICUs are highly medical and technological environments where VPT infants are exposed to maternal separation and different sources of physical (lights and sounds, invasive (ventilation) and painful (skin-breaking procedures) stimulations (Greisen et al., 2009; ; Grunau et al., 2006; Grunau, 2013; Brummelte et al., 2012).

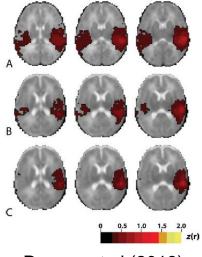
NICU-related care

Developmental Care (DC) interventions (Als, 2011; Flacking et al, 2012; Welch et al, 2014) are family-centered strategies meant to promote parental involvement, early physical contact, emotional bonding, breast-feeding, infants' neurobehavioral stability and parenthood transition.

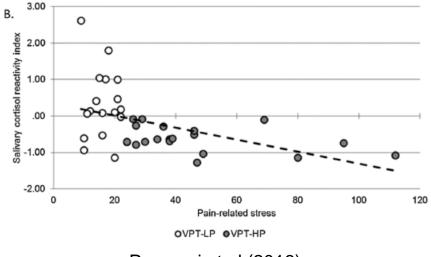


Pain-related stress exposure in NICU Effects on brain and neuroendocrin





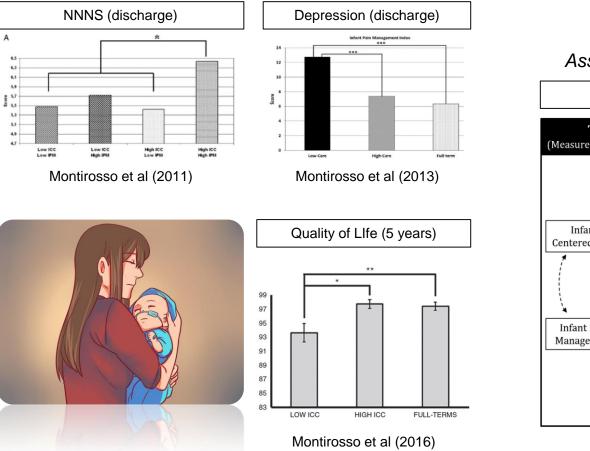
Ranger et al (2013)



Provenzi et al (2016)

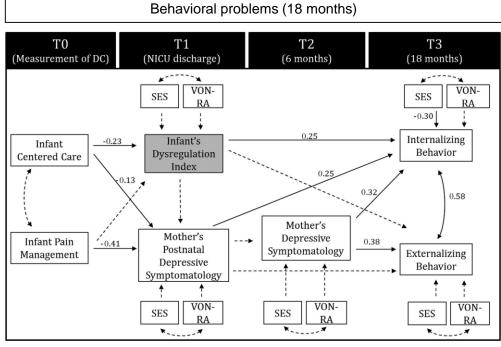
Developmental Care effects

Data from multi-centric study (k = 25)



The Neonatal Adequate Care for Quality of Life (NEO-ACQUA) study

Assessment tool: Quality of Care Checklist [quantitative]



Montirosso et al (2017)

The missing link How do NICU exposures leave a trace...?

NICU-related stress

But which are the mechanisms linking early NICU-related exposures (for bad and for good) with developmental trajectories and phenotypic outcomes?

HPA axis stress dysregulation (Provenzi et al., 2016).

Poor neurobehavior at discharge (Lester et al., 2011).

Higher risk of behavioral problems (Chau et al., 2014).

Altered brain growth (Ranger et al., 2015; Smith et al., 2011).

NICU-related care

Better HPA axis stress regulation (Kleberg et al., 2008).

Better neurobehavioral profile at discharge (Montirosso et al., 2012).

Reduced risk of behavioral problems (Montirosso et al., 2018). Better brain growth (Als et al.,

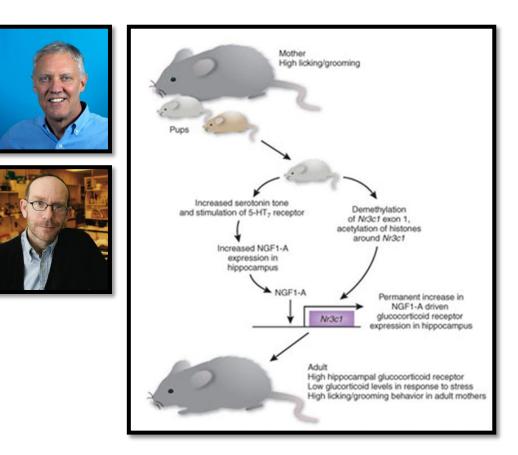
2004).



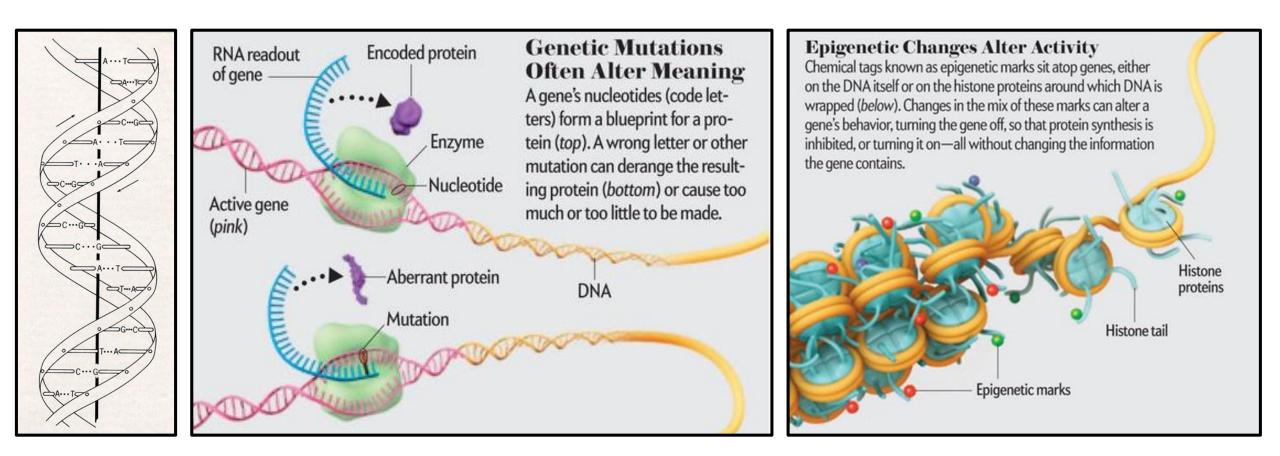
What makes a good rat mama

How the care environment gets under the skin

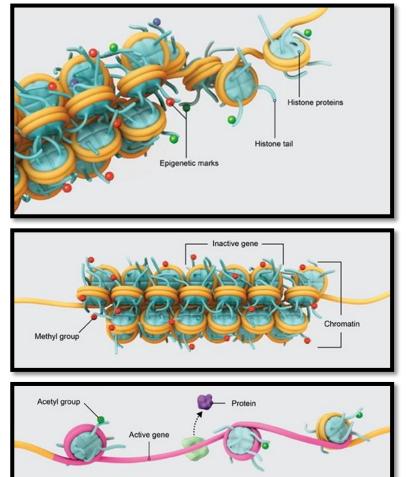


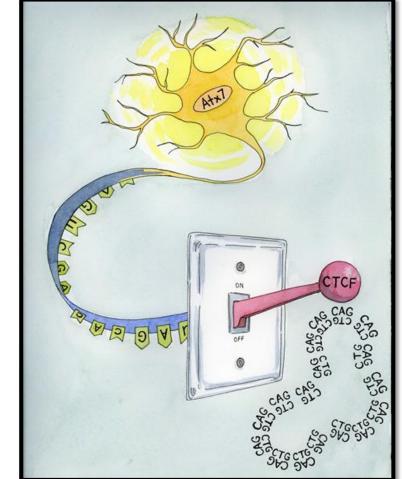


More than your genes Why your DNA is not your destiny



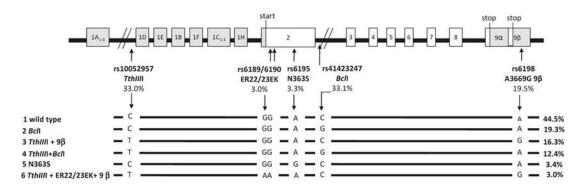




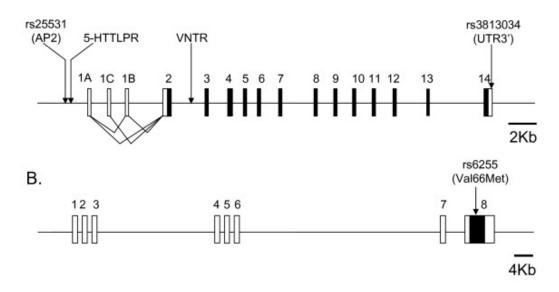




Methylation changes in specific genes Candidate loci of environmental susceptibility?



Α.



NR3C1

Gene encoding for the glucocorticoid (cortisol in human) receptors in the brain. Cortisol is involved in developmental regulation of.

- Cognitive functions
- · Memory formation and consolidation
- Emotional behavior
- · Stress response and reactivity

SLC6A4

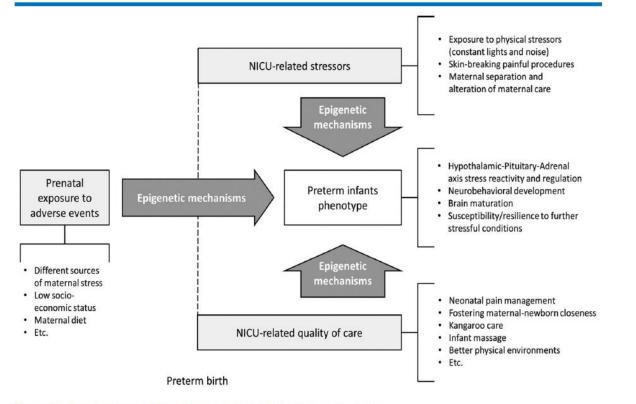
Gene encoding for the serotonin transporter. Serotonin transporter are involved in developmental regulation of:

- Cognitive functioning
- Memory consolidation
- Emotional behavior
- Stress regulation



Preterm Behavioral Epigenetics

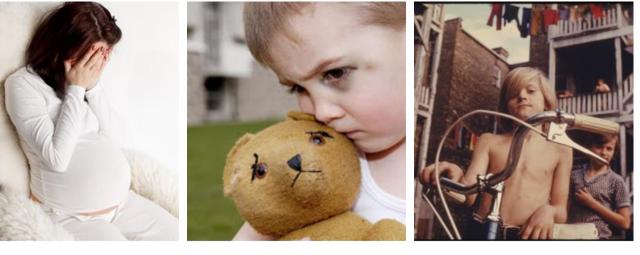
Theoretical model and rationale

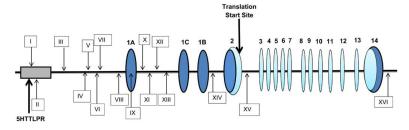


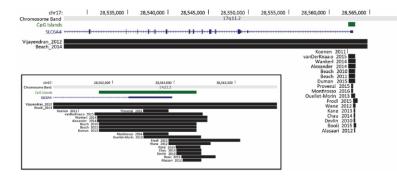
JOGNN, 44, 174-182; 2015. DOI: 10.1111/1552-6909.12559 Implications of Epigenetics and Stress Regulation on Research and Developmental Care of Preterm Infants Rosario Montirosso and Livio Provenzi



SLC6A4 methylation Biomarker of adversities in humans







Provenzi et al (2017)

Devlin et al., 2010

Duman & Canli, 2011

Essex et al., 2013

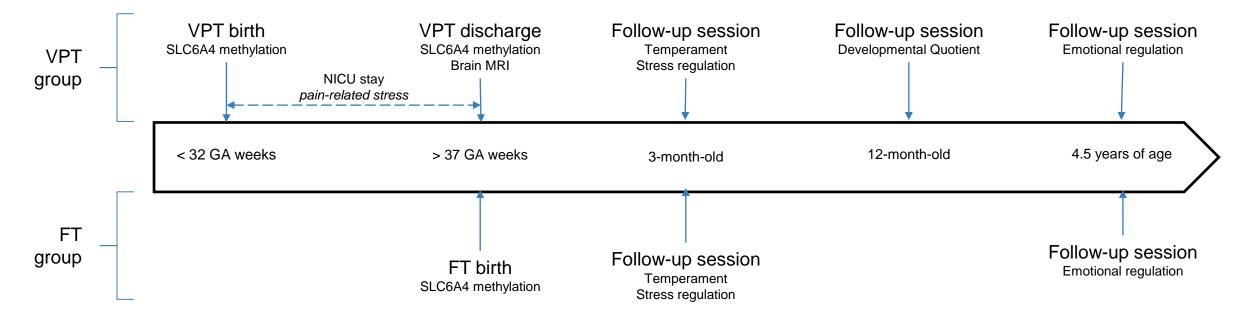
The PBE project Study design and aims



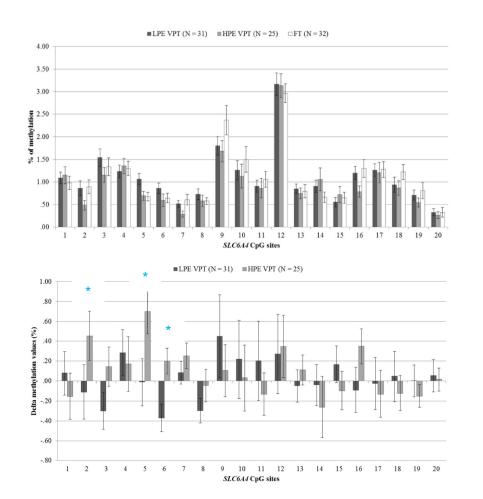
n = 64 VPT infants (excluding major neonatal and perinatal complications)



n = 56 Full-term (FT; gestational age > 37 weeks) healthy infants



Results [1] Effects of early pain-related stress on SLC6A4 methylation in VPT infants



frontiers in Behavioral Neuroscience

Pain-related stress during the Neonatal Intensive Care Unit stay and *SLC6A4* methylation in very preterm infants

Livio Provenzi¹, Monica Fumagalli², Ida Sirgiovanni², Roberto Giorda³, Uberto Pozzoli⁴, Francesco Morandi⁵, Silvana Beri³, Giorgia Menozzi⁴, Fabio Mosca², Renato Borgatti⁶ and Rosario Montirosso^{1*}

VPT infants split in low (LPE) and high (HPE) pain exposure based on median value of skinbreaking procedures count.

Aim #1: no differences in SLC6A4 CpG-specific methylation at birth among VPT-LPE, VPT-HPE and FT infants.

Aim #2: Significant birth-to-discharge increase in SLC6A4 methylation only in VPT-HPE infants.

Results [2] Effects of SLC6A4 methylation increase on temperament at 3 months

CHILD DEVELOPMENT

Serotonin Transporter Gene (*SLC6A4*) Methylation Associates With Neonatal Intensive Care Unit Stay and 3-Month-Old Temperament in Preterm Infants

Rosario Montirosso and Livio Provenzi IRCCS Eugenio Medea Monica Fumagalli and Ida Sirgiovanni University of Milan

Roberto Giorda, Uberto Pozzoli, Silvana Beri, and Giorgia Menozzi IRCCS Eugenio Medea

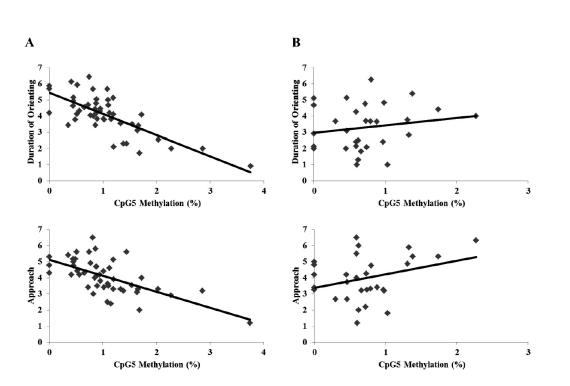
> Francesco Morandi Sacra Famiglia Hospital

Ed Tronick University of Massachusetts and Division of Newborn Medicine

> Fabio Mosca University of Milan

Renato Borgatti IRCCS Eugenio Medea

Aim #3: increased CpG-specific SLC6A4 methylation at discharge linked with less orienting and approach in VPT infants. No significant associations in FT infants.





Results [3]

Long-term effects: Developmental Quotient at 12 months *via* brain alterations

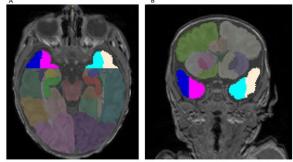
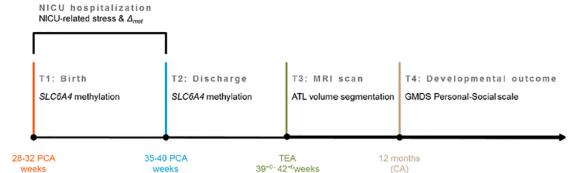


Fig 2. Brain MRI segmentation: A. axial and B. coronal view T1 images. Note. Colors highlight anterior temporal lobe (ATL) lateral part left (ATL-LPL, yellow) and right (ATL-LPR, dark blue) as well as ATL medial part left (ATL-M.PL, light blue) and right (ATL-MPR, pink).

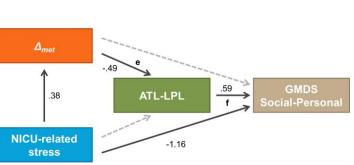


From early stress to 12-month development in very preterm infants: Preliminary findings on epigenetic mechanisms and brain growth

Monica Fumagalli^{1e}, Livio Provenzi^{2e}, Pietro De Carli², Francesca Dessimone¹, Ida Sirgiovanni¹, Roberto Giorda³, Claudia Cinnante⁴, Letizia Squarcina⁵, Uberto Pozzoll⁶, Fabio Triulzi⁴, Paolo Brambilla^{5,7}, Renato Borgatti⁸, Fabio Mosca¹, Rosario Montirosso²*

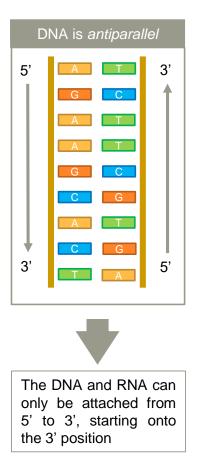


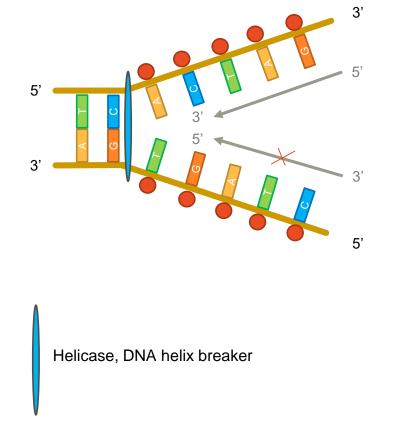
A Amet .64 .50 .38 ATL-MPL b ocial-Persona **NICU-related** -1.40 stress B ∆_{met} -.56 .50 .38 ATL-LPR ocial-Persona d **NICU-related** -1.34 stress С Δ_{met}

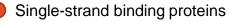


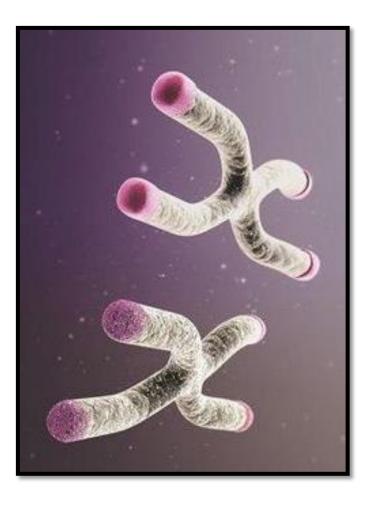
More than methylation

The anti-parallel DNA and telomere issue



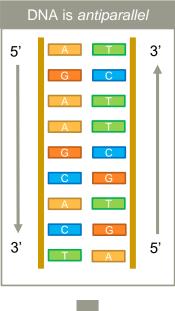


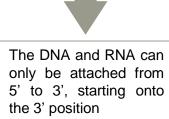


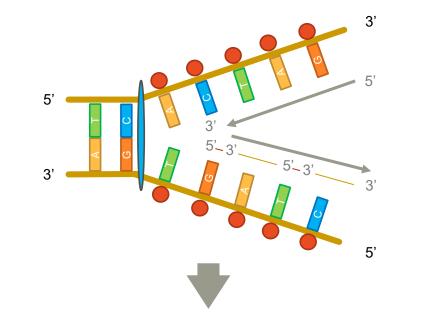


More than methylation

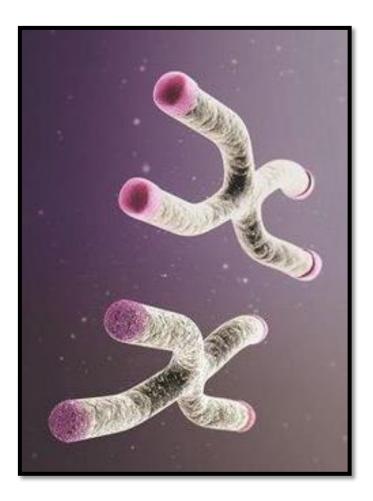
The anti-parallel DNA and telomere issue





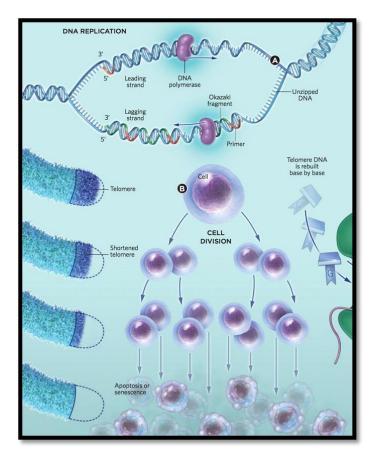


The DNA Okazaki fragments are primed by an RNA primer, which is finally replaced with DNA by a replacement enzime. The fragments are binded by a ligase enzyme. Nonetheless, the final gap will be not binded and a specific series of nucleotides will not copie and it will be lost.





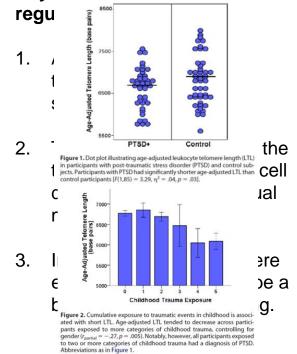
Telomere length (TL) erosion Biomarker of cellular aging and stress



BIOL PSYCHIATRY 2011;70:465-471

Childhood Trauma Associated with Short Leukocyte Telomere Length in Posttraumatic Stress Disorder

Aoife O'Donovan, Elissa Epel, Jue Lin, Owen Wolkowitz, Beth Cohen, Shira Maguen, Thomas Metzler, Maryann Lenoci, Elizabeth Blackburn, and Thomas C. Neylan



PNAS | August 16, 2011 | vol. 108 | no. 33 | E513-E518

Stress exposure in intrauterine life is associated with shorter telomere length in young adulthood

Sonja Entringer^a, Elissa S. Epel^b, Robert Kumsta^c, Jue Lin^d, Dirk H. Hellhammer^a, Elizabeth H. Blackburn^d, Stefan Wüst¹, and Pathik D. Wadhwa^{sg,1}

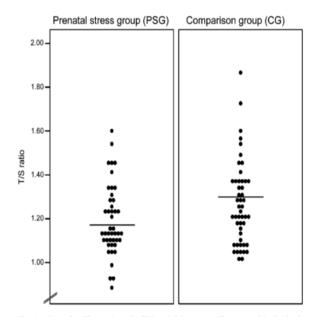


Fig. 1. Dot plot illustrating LTL (T/S ratio) in prenatally stressed individuals (PSG; n = 45) and CG subjects (n = 49). Lines indicate group means.

PBE project + telomeres

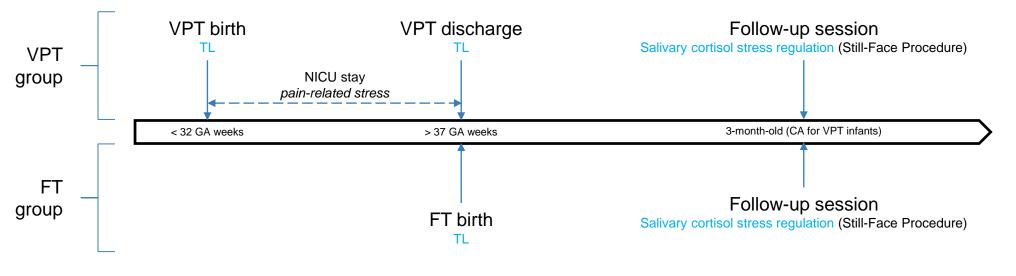
Recap of study design and TL-related aims



n = 46 VPT infants (excluding major neonatal and perinatal complications)



n = 31 FT healthy infants

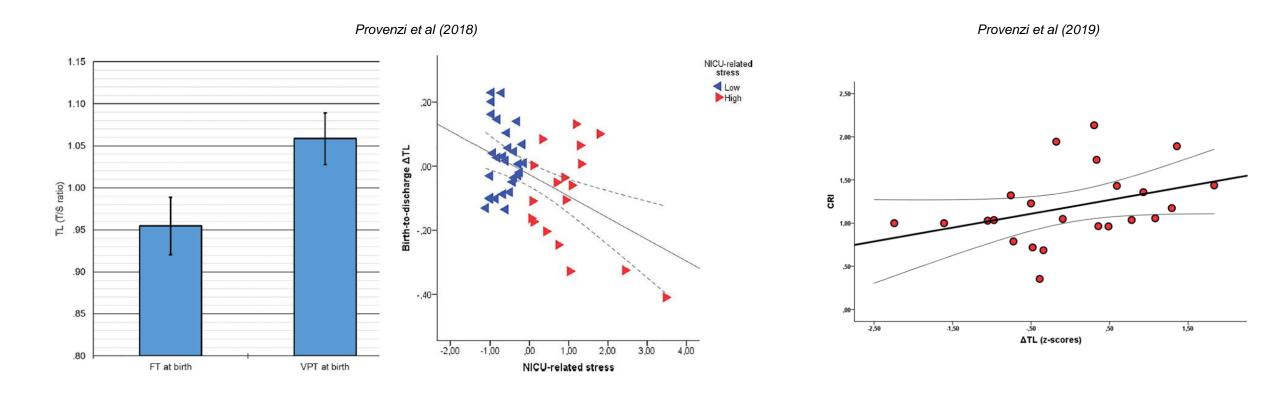


Aim 1: birth-related TL difference between VPT and FT infants?

Aim 3: TL effect on VPT and FT infants salivary cortisol stress regulation?

Aim 2: pain-related effect on TL in VPT infants?

PBE & TL From NICU to HPA axis programming



Behavioral Epigenetics

Why should we be interested in this stuff?



If they ask you anything you don't know, just just say it's due to epigenetics.

What Behavioral Epigenetics can give to us

- 1. Better understanding of early life exposures
- 2. Unveiling biological underpinnings of developmental

programming

- 3. Contributing to evidence-based support for early interventions
 - 4. Supporting complex view of human development
 - 5. Advancing the nature vs. nurture field of studies

What we can give to Behavioral Epigenetics

- 1. Guiding the wide-spread of research in the field
- 2. Providing relevant questions for clinical issues
- 3. Providing interpretations of findings in context
- 4. Investing properly research funds into relevant directions
- 5. Integrating multiple epigenetics biomarkers in a

developmentally oriented way



NICU-related epigenetic regulation Emerging linking mechanisms

1. From NICU to DNA

2. From DNA to outcome

3. What's next?

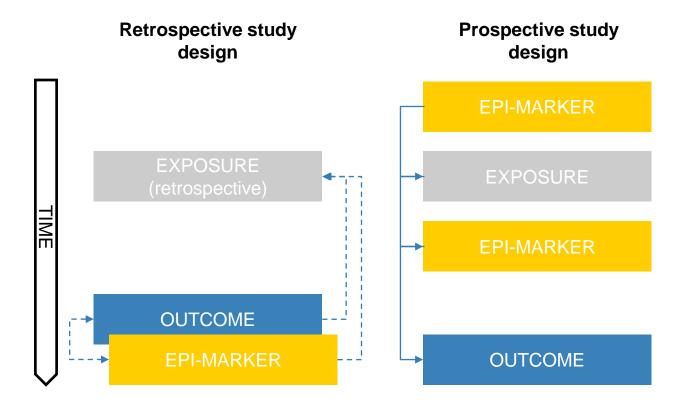
NICU-related stress associates with environmental-driven regulation of methylation status of specific gene associated with behavioral and socio-emotional development (SLC6A4) and TL shortening. Increased SLC6A4 methylation and TL shortening associate with differential less-thanoptimal outcomes in VPT infants, including behavioral difficulties, socio-emotional stress hyper-reactivity, and HPA axis dysregulation. We are looking forward to

- A. Long-term trajectories up to 4 years of age;
- B. Epigenetic correlates of DC practices;
- C. Epigenetics of neural mirroring system in VPTs;
- Key role of NICU touch in VPT infants' epigenetic regulation.

Be

Behavioral epigenetics in VPT infants

Study design advantages



Subjective report bias

- Correlational, not predictive
- · Limited moderation analysis
- Limited control of confounders

- Less subjective report bias
- Causal links possible
- Supports moderation analysis
- Supports control of confounders

JAMA Pediatrics

Why Are Prospective Longitudinal Studies Needed in Preterm Behavioral Epigenetic Research?

Livio Provenzi, PhD Renato Borgatti, MD Rosario Montirosso, PsyD

Author Affiliations: 0-3 Center for the At-Risk Infant, Scientific Institute Eugenio Medea, Bosisio Parini, Italy (Provenzi, Montirosso); Neuropsychiatry and Neurorehabilitation Unit, Scientific Institute Eugenio Medea, Bosisio Parini, Italy (Borgatti).

JAMA Pediatrics

"Epigenethics" in the Neonatal Intensive Care Unit Conveying Complexity in Health Care for Preterm Children Livio Provenzi, PsyD Rosario Montirosso,

JAMA Pediatrics

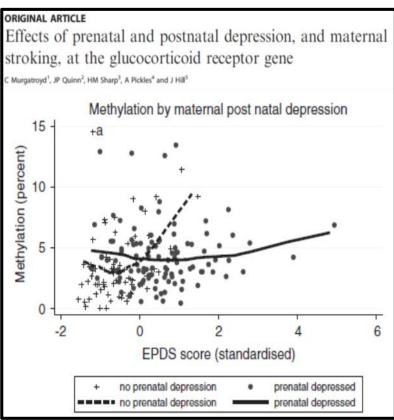
Behavioral Epigenetics of Family-Centered Care in the Neonatal Intensive Care Unit

Livio Provenzi, PsyD Serena Barello, PhD



What about resilience?

Epigenetics and protective factors

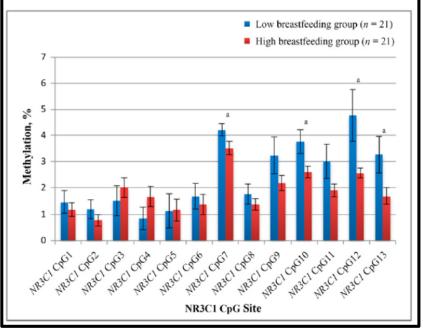


maternal touch

maternal breastfeeding

Epigenetic Programming by Maternal Behavior in the Human Infant

Barry M. Lester, PhD, a,b,c Elisabeth Conradt, PhD,d Linda L. LaGasse, PhD, a,b,c Edward Z. Tronick, PhD,^e James F. Padbury, MD,^{b,c} Carmen J. Marsit, PhD^f



Ongoing now

Epigenetic vestiges of an early parental intervention using video-feedback



	T0 – Baseline	T1 – Post- Intervention	T2 – FollowUp+3m	T3 – FollowUp+6m
(EXP GROUP)	(10 min. GL 10 min DFFSF (8 weekly session)	10 min. GL 10 min DFFSF	10 min. GL 10 min DFFSF	10 min. GL 10 min DFFSF
(CTR GROUP)	10 min. GL 10 min DFFSF (weekly session)	10 min. GL 10 min DFFSF	10 min. GL 10 min DFFSF	10 min. GL 10 min DFFSF
Layer 1:	(M behavior)	(M behavior)	(M behavior)	(M behavior
behavior	I behavior		I behavior)	I behavior
Layer 2:	Oxytocin	Oxytocin	Oxytocin	Oxytocin
hormones	Cortisol	Cortisol	Cortisol	Cortisol
Layer 3: epigenetics	Methylation OXTR, SLC6A4, BDNF, NR3C1	Methylation OXTR, SLC6A4, BDNF, NR3C1	Methylation OXTR, SLC6A4, BDNF, NR3C1	Methylation OXTR, SLC6A4, BDNF, NR3C1
Layer 4: self-report	BDI, STAI, PSI	BDI, STAI, PSI	BDI, STAI, PSI	BDI, STAI, PSI
	CBCL, IBQ	CBCL, IBQ	CBCL, IBQ	CBCL, IBQ
Layer 5:	Engagement	Engagement	Engagement	Engagement
process	Acceptability	Acceptability	Acceptability	Acceptability

www.livioprovenzi.com

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Fabio Mosca Monica Fumagalli

Pediatric Unit, Fatebenefratelli, Erba

Francesco Morandi











